No Postage Required: Extracellular Vesicles Deliver the Message

[an Editorial for the Theme "Extracellular Vesicles in Cell Physiology"]

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Extracellular vesicles (EVs) were first appreciated when electron microscopists illuminated the structure of cells and the materials they released into the extracellular space (9). However, the number of publications related to EVs hovered below two hundred per year from the late 1970's until 2008. The last 10 years have witnessed a 10- to 20-fold increase in the number of publications on EVs per year.

The ability of these structures to capture the attention of scientists comes, in part, from the important roles in cell communication they have been found to play and the diverse array of processes they are involved in. EVs are ubiquitous to life and are made by uni- and multi-cellular life forms including eukaryotes such as yeast (6) and parasites and prokaryotes such as bacteria and archaea (3). Also, viruses interact with EVs, and EVs appear to utilize viral cell entry pathways to cells (10). Thus, EVs are a highly conserved cellular adaptation, and eukaryotic organisms share proteins that regulate EV formation such as the endosomal sorting complexes required for transport (4). EVs originate by direct cell membrane budding or from intracellularly generated bodies containing multiple vesicles that fuse with the cell membrane. In either mechanism, the EVs escape into the extracellular space and have potential to bind local or distant cells. While details of these biogenic pathways have been described (1), it is likely that other mechanisms remain to be discovered. EVs are packed with a range of bio-reactive materials including several forms of RNA, mitochondria (5), lipids, enzymes, second messenger cyclic nucleotides (8), and metabolites, which are released by EVs, upon membrane fusion, into the cytoplasm of target cells. Further, EVs are decorated with proteins that reflect the surface expression of the parent cells (7) suggesting that EVs may signal by intersecting with established ligand-receptor mechanisms.

As with any burgeoning area of scientific investigation, the EV field has suffered from confusion in terminology, classification, methods of isolation and preparation and a paucity of details in experimental protocols, amongst other things. This has resulted in heterogeneity in published findings and little experimental reproducibility. Stimulated by a number of EV-focused professional groups and publications, progress is being made in correcting these deficiencies. Efforts to standardize definitions and terms, harvesting and processing protocols, and the application of the same to GMP programs is being made. This is needed given the expanding number of EV-focused clinical trials. A recent search of ClincalTrials.gov employing the term 'extracellular vesicles' identified at least a dozen trials. The identified trials explore the biology, biomarker and therapeutic applications of EVs.

Consideration of issues surrounding EV research design, reporting and clinical trials highlights areas for improvement:

- *In vivo* demonstration of EV formation, movement, lodgment and uptake should be undertaken. A strategy to characterize the *in vivo* physiologic and pathologic parameters that govern EV activities over the life cycle is paramount. This is necessary if any therapeutic potential is to be realized.
- Activities of EVs upon established non-EV signaling pathways need to be tested. As a 'Johnny come lately' field within the cell biology realm, there are important questions on what aspects of canonical cell signaling are impacted by EV-related mechanisms. Do EVs shape canonical ligand-receptor interactions or *vice versa*? What parameters set the playing field: that is, which signaling mechanism dominates under physiological

conditions? Do therapies/drugs that target standard ligand-receptor interactions alter EV signaling and do EVs alter the therapeutic effect of these drugs?

- In relation to further research, the most appropriate EV-relevant control agents and parameters/standards need to be identified and initiated in cell and animal studies.
- Further enquiries into the interaction between EVs and other agents administered to cell cultures will likely prove of interest. In this regard, could EVs be accountable (in part) for variability in standard cell culture experimental designs? As a 'contaminant' in a biologic preparation, do they hitch a ride and either direct or modify the outcomes as an unaccounted component of agents given to cells or whole organisms? Can we be sure that other GMP-produced biologics do not include EVs that survive the production process? Thus, could the therapeutic result of these biologics be (at least in part) an effect of EVs?
- As government bodies continue to receive clinical trial requests from academic and industry teams seeking to determine whether EVs have healing properties, a step back may be reasonable. Large, well-controlled and blinded clinical studies looking to determine associative, causative, or contributive niches held by EVs in diseases, trauma and health should be started with translation across ethnic, economic and geographic boundaries. GMP production and clinical study minimum guidelines should be determined and invoked.
- Also important for future research and publications would be the development of rigorous isolation, identification, characterization and confirmative protocols agreed upon by an international consensus of researchers and governmental bodies with sustained activity in the EV field. These can then be promulgated and accepted by major scientific bodies and journals. As major international scientific publishers have established and aligned

themselves behind a minimum threshold of scientific consistency in general methods and reporting, this rigor should also be applied to reporting of EVs. Unification of terminology and classification of EV and EV-associated particles into categories defined by physical properties and mode of genesis should be achieved in oral and written scholarly communications. Although future discoveries may require revision of the initial organizational/classification systems, starting a discussion soon will be of value. The current arbitrary aspects have opened up the field to constant criticism and made objective interpretation of data daunting. Addressing these matters fits well with the laudable efforts to improve scientific rigor, transparency and reproducibility in general and especially in the physiological sciences, in which the American Physiological Society is playing an active part.

Biologics have a 'favored child' position in the therapeutic realm and for individuals may provide treatment for what were previously undruggable diseases. EVs are passively benefiting from the special accommodations currently afforded biologics (e.g. stem cells). However, until defined classification, processing, and validation issues are resolved, any benefits ascribed will likely be associative.

In the present Theme on "Extracellular Vesicles in Cell Physiology" the current state of EV research is reviewed by a number of leading groups with a focus on EV involvement in the areas of stem cell biology, leukemia, and tumor progression. In this issue, the Theme begins with a Review by Dr. Borgovan and colleagues on EVs in leukemia (2).

The Editors of the American Journal of Physiology - Cell Physiology thank all of the authors for their time and effort in contributing these excellent Reviews. We hope that readers will find

these articles of interest and a stimulus to consider the possible roles of EVs in their own experimental systems. We cordially invite all investigators to submit research articles for a Call for Papers on "Extracellular Vesicles in Cell Physiology" which will open for submissions on June 1, 2019.

Conflict of Interest: J.S.I. serves as Chief Science Officer for Radiation Control Technologies, Inc. and is co-inventor of an NIH patent licensed for development by the same. J.C.A declares no conflicts of interest.

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